Rapid identification of OXA-23-subfamily in carbapenem-resistant *Acinetobacter* spp. with a novel immunochromatographic lateral flow assay

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CORIS BioConcept

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Sonja Mertins[1,2], Paul G. Higgins[1,2], Laurence Denorme[3], Quentin Gilleman[3], Pascal Mertens[3], Harald Seifert[1,2], Martin Krönke[1,2] and Alexander Klimka[1,2]

[1] Institute for Med. Microbiology, Immunology and Hygiene, University Hospital of Cologne, Goldenfelsstr. 19-21, 50935 Cologne, Germany; [2] German Centre for Infection Research, partner site Bonn-Cologne, Germany; [3] Science Park CREALYS, Rue Jean Sonet 4A, B-5032 Gembloux, Belgium

Introduction

The global spread of carbapenem-resistant *Acinetobacter* spp. has led to an emerging worldwide healthcare problem. The carbapenem-hydrolysing oxacillinases (OXAs) are the most commonly reported carbapenem-resistance determinants in *Acinetobacter* spp., particularly in *A. baumannii*. There are six identified OXA-subgroups associated with carbapenem-resistance in *A. baumannii*: the intrinsic OXA-51-like and the acquired OXA-23-like, OXA-58-like, OXA-40-like, OXA-143-like and OXA-235-like. Of these, OXA-23 is the most prevalent carbapenem-resistance determinant among isolates in Germany, Europe and worldwide.

The lack of effective and reliable tests to detect OXA-mediated carbapenem-resistance is a serious challenge to modern medicine. There is an unmet medical need for reliable and rapid diagnostic tools to detect OXA-23-like producing strains to ensure a successful treatment of patients and prevent the spread of carbapenemase-producers.

The aim of this work is the development an antibody-based OXA-23-like

Results

Selection of hybridoma clones

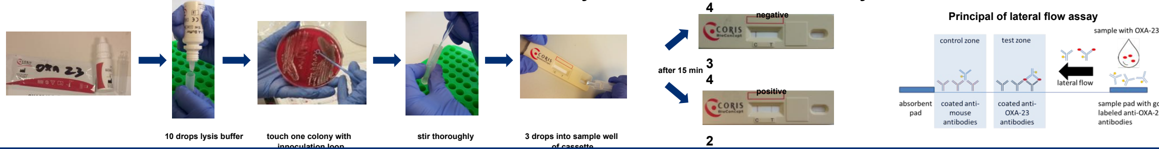
Mice were immunized with purified recombinant OXA-23$_{6xHis\text{-}Tag}$. After a standardized immunization protocol, one mouse was sacrificed, spleen was processed, and generated splenocytes were fused with myeloma cells to generate hybridomas.

900 163 156 42

- hybridoma clones were picked into 96-well plates
- clones exhibit OXA-23$_{6xHis\text{-}Tag}$ specificity
- clones exhibit OXA-23$_{6xHis\text{-}Tag}$ specificity, excluded 6xHis-Tag binders
- clones exhibit OXA-23$_{6xHis\text{-}Tag}$ specificity after adaptation to serum-free medium
- clones exhibit OXA-23$_{6xHis\text{-}Tag}$ specificity, tested for cross reactivity to other OXA-sub groups
- clones exhibit OXA-23 specificity, binding to OXA-23 from *A. baumannii* lysate
- clones secrete antibodies showing a balanced heavy-/light-antibody-chain ratio in SDS-PAGE
- clones were selected for mass production, purified antibodies were screened in prototype setting
- **remaining suitable clones**

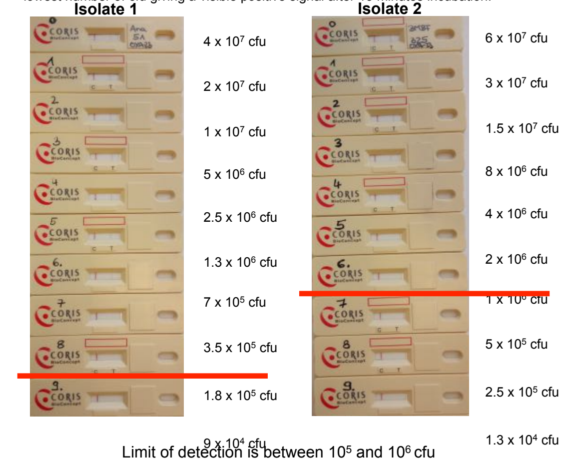
24 34 2 21 2-4

Flow chart of antibody-based OXA-23 detection assay



Sensitivity of OXA-23 detection kit

To determine the limit of sensitivity of the prototype, overnight cultures of OXA-23-producing carbapenem-resistant *Acinetobacter baumannii* isolates were 2-fold diluted in lysis buffer, and lysates were applied to the test strips . In parallel, we plated out serial dilutions of the culture to determine the cfu's. The detection limit was defined as the lowest number of cfu giving a visible positive signal after 15 minutes incubation.



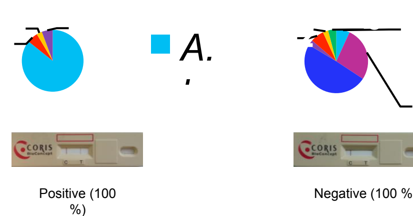
Limit of detection is between 10^5 and 10^6 cfu

Specificity of OXA-23 prototype

A well characterized collection of carbapenem-resistant *Acinetobacter* spp. isolates (n=108) with defined carbapenem resistance mechanism were used to evaluate specificity of our OXA-23 prototype.

Acinetobacter spp. (n= 108)	Carbapenemase gene	Positive	Negative
***A. baumannii* (IC1-IC8) (n= 96)**	*bla*$_{OXA\text{-}23\text{-}like}$ (n= 30)	30	0
	bla$_{OXA\text{-}40\text{-}like}$ (n= 19)	0	19
	IS*Aba1-bla*$_{OXA\text{-}51\text{-}like}$ (n= 5)	0	5
	bla$_{OXA\text{-}58\text{-}like}$ (n= 32)	0	32
	bla$_{OXA\text{-}143\text{-}like}$ (n= 1)	0	1
	bla$_{OXA\text{-}235\text{-}like}$ (n= 5)	0	5
	bla$_{VIM\text{-}2}$ (n= 1)	0	1
	bla$_{NDM\text{-}1}$ (n= 2)	0	2
	bla$_{NDM\text{-}2}$ (n= 1)	0	1
***A. pittii* (n= 8)**	*bla*$_{OXA\text{-}23\text{-}like}$ (n= 2)	2	0
	bla$_{OXA\text{-}40\text{-}like}$ (n= 1)	0	1
	bla$_{OXA\text{-}58\text{-}like}$ (n= 3)	0	3
	bla$_{OXA\text{-}143\text{-}like}$ (n= 1)	0	1
	bla$_{VIM\text{-}2}$ (n= 1)	0	1
***A. nosocomialis* (n= 2)**	*bla*$_{OXA\text{-}23\text{-}like}$ (n= 1)	1	0
	bla$_{OXA\text{-}58\text{-}like}$ (n= 1)	0	1
***A. radioresistens* (n= 2) (carb^R strains with intrinsic OXA-23 associated with IS*Acra*1)**	*bla*$_{OXA\text{-}23\text{-}like}$ (n= 2)	2	0

OXA-23-producer (carb^R) **Other c**



Positive (100 %) Negative (100 %)

Results of ten representative test strains

Conclusions

- no expensive or specialized equipment is required to use this test strip
- antibody-based OXA-23 detection assay is able to detect OXA-23-mediated carbapenem-resistant *Acinetobacter* spp. with 100% specificity
- sensitivity has been determined to detect between10^5 to 10^6 cfu per sample, making a point-of-care device feasible
- result in < 20 min which saves 12-48 hours in diagnostic time, avoiding treatment with inappropriate antibiotics and enables earlier intervention to control transmission of OXA-23 producing carbapenem-resistant *Acinetobacter* spp.

email: aklimka@uni-koeln.de